Abstract

The invention is directed to a method for sequencing multiple target polynucleotide segments in parallel, and to compositions and kits therefor. In the method, a plurality of sample polynucleotide fragments are used to form a mixture of different-length sequencing fragments. The sequencing fragments are complementary to at least two different sample fragments, wherein (1) each sequencing fragment terminates at a predefined end with a known base or bases, and (2) each sequencing fragment contains an identifier tag sequence that identifies the sample fragment to which the sequencing fragment corresponds. The sequencing fragments are then separated on the basis of size to produce a plurality of resolved, size-separated bands. Resolved bands are collected in separate aliquots, which, in a preferred embodiment, are then subjected to an amplification step to amplify the complements of the tag sequences in each aliquot, and preferably, the tag sequences too. Amplification is preferably by PCR. The (amplified) aliquots are then separately hybridized with an array of immobilized different-sequence tag probes under conditions effective to provide specific hybridization of tag sequences, or of tag sequence complements, with the corresponding immobilized tag probes, to form a hybridization pattern on the array, from which sequence information of one or more sample fragments are determined.